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New Views on Rejection Mechanisms and Their Relevance to Interleukin-2 as a Treatment for Renal Cell Cancer

R.T.D. Oliver

IT IS now nearly a century since the first efforts to cure cancer by tumour vaccination [1]. Most of these early efforts only helped to understand the basis of allogenic transplantation rejection [2], a paradox given that today these antigens are now proving to be of prime importance to understanding tumour rejection. Once the genetic basis of major histocompatibility (MHC) systems had been worked out [3], it was possible to demonstrate that rejection of syngenic tumours was also mediated by immune response [4] and immune memory rests in T-lymphocytes [5], as does memory for graft rejection [6].

The 1970s saw many attempts to apply the findings from animal studies to the clinic [7]. Unfortunately, most of this work failed because it used treatments with unproven effect in the measurable disease setting in patients without measurable disease [though researchers in adult solid cancer realised this error when they came to study interleukin-2 (IL-2), it is unfortunate that the same mistake is being made today in the field of leukaemia: IL-2 is being used as adjuvant after marrow transplantation without any evidence from dose-response studies in patients with measurable disease]. Only at the end of the 1970s, when interest in the idea was fading because of lack of obvious clinical benefit, did the first clues as to how to induce specific anti-autologous human tumour immune response appear. These came from understanding the differences in the molecular basis of antigen presentation to helper (HLA class II-dependent) and cytotoxic (HLA class I-dependent) T-lymphocytes. Differences of both class of antigen had to be present (though not necessarily on the same cell) to induce an effective transplantation rejection response [7] while matching of target and effector cell for these same antigens proved necessary for effective antiviral [9] and antitumour immune response [10].

The first clue that these observations might be relevant to human malignancy came from the study of adults with acute myeloid leukaemia in remission [11–13]. These studies demonstrated that the pretreatment leukaemic blast cells, though expressing more serologically detectable class II antigen than remission lymphocytes, failed to provide an effective stimulus to provoke either an autologous or allogenic cytoxic T-lymphocyte response [11]. However, when the autologous blasts were presented to the remission lymphotyces in association with an allogenic class II antigen cytotoxic, T-lymphocytes with specificity restricted to the autologous leukaemic blast were produced [12, 13]. Recently studies in melanoma and colon cancer have provided even more convincing evidence that loss of functional class II antigen occurs quite frequently as a mechanism of escape from immune surveillance. In melanoma, Alexander *et al.* [14] have demonstrated that cell-lines developed from metastatic melanoma, though expressing excess serologically detectable class II antigens, failed to function in an assay of antigen presentation to helper cells. The same group was able to demonstrate that the defect could be overcome by transfecting a normal class II DR beta gene into the metastatic cell line [15].

Additional support for the importance of class II came from

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the studies of Ranson *et al.* in colon cancer [16]. They showed that success in their trial of vaccine therapy using autologous tumour combined with BCG depended on the presence of induceable class II on the tumour cells being used as a vaccine. Supportive evidence for this observation comes from studies in melanoma by Cohen *et al.* [17] who demonstrated that response of metastases to IL-2 depended on presence of induceable class II antigen on the tumour.

This observation, although based on a small number of patients, almost certainly provides the most plausible explanation for how IL-2 is able to produce such impressive durable complete remissions in human tumours, albeit in such a small minority of patients with renal cell and melanoma, the only two tumours to be investigated in depth [18]. The principal role for IL-2 in the immune response is to act as an autocrine growth factor to amplify helper cells and in a paracrine manner to recruit and amplify cytotoxic T cells [19]. As such, it may well be able to compensate partly for a lack of functioning class II antigen on the tumour cells providing class I antigen is present in sufficient quantity to present the weak tumour antigen to the cytotoxic T-lymphocyte (CTL).

Support for this view comes from the study of tumour infiltrating lymphocytes (TILs) from melanomas. These show that it is possible to use IL-2 to expand them in culture from suspensions of tumour cells. About 40% of patients produce HLA class I restricted CTL [20, 21] which have oligoclonal T-cell receptor phenotype [21, 22] and when expanded and returned to the patient with a neomycin-resistance gene marker could be demonstrated in the circulation for up to 189 days and in tumour up to 64 days after injection [23]. In addition they produced remissions in 38% of patients [24].

For renal cell cancer, the evidence for immunological specificity induced by IL-2 is less clearcut and most of the TIL cells generated *in vitro* show broadly reactive non-HLA-restricted NK/LAK-like cytotoxicity. These are the sort of cells that predominate in the nude mouse, which lacks a functional thymus [25], and are the first lymphocytes to appear in the adult after total body irradiation and bone marrow transplantation without thymic replacement [26]. Little work has been done on thymic function in cancer patients, though the average age of melanoma patients is 10–20 years younger than renal cell cancer patients and thymic function declines with age. Investigation of the effect of thymic hormones on TIL cell specificity could well be worth further investigation. Support for this idea comes from data in mice demonstrating that thymic hormones are capable of converting broadly reacting non-MHC-restricted cytotoxic cells into MHC-restricted specific cells [27].

There is an additional factor that could be involved in the lack of specificity of TILs from most adult solid tumours other than melanoma. This relates to the frequency with which defects of class I antigen expression can be detected on all adult solid tumours studied to date, such as bowel [28], bladder [29, 30] and pancreas tumours [31]. Such defects would of course lead to inability of the cytotoxic T-cells to recognise weak tumour antigens. Of course this defect could not be overcome by IL-2, though if relative rather than absolute it might be compensated for by the effect of alpha interferon (IFN-α), which augments expression of class I antigens [32]. The complementary actions of IL-2 and IFN-α have provided the justification for studies of the two cytokines in combination.

Though there is strong evidence from animal studies that the two cytokines are additive when used in combination [33, 34], to date the results from clinical trials are conflicting. Though some of the earlier studies produce response rates in excess of 30% compared with single-agent responses of 15%, a review of more than 200 patients [35] showed overall responses of 21%. This compares with 15% for single-agent IL-2 and 14% for single-agent IFN-α [36] and the only formal randomised trial showed worse reponse for the combination than for the single agents [37]. Most of these were small pilot studies and few have provided extensive dose-response escalation. The only trial to do so showed a bell-shaped dose response with lower responses at high compared with intermediate doses [38]. In view of this, it may be that the optimum dosing schedule has yet to be defined.

It would seem that IL-2 is a key factor in the immunology of resistance to tumour. In the subset of tumours where there are class II-based antigen presentation defects but normal class I antigens, IL-2 could well play a particularly important therapeutic role. The recent demonstration that tumours arising in immunosuppressed individuals (either after transplantation or HIV infection) are more likely to have normal HLA antigen expression on their surface than spontaneously occurring tumours [39], suggests that this group of tumours might be particularly sensitive. Clinical information in this area is sparse, though it is clear that results of conventional immunosuppressive chemotherapy treatment of lymphoma is often worse in tumours from the immunosuppressed group than in the spontaneously occurring tumours.

Despite all of this potential and experience from treating more than 500 patients, some of whom have had durable complete remission now lasting beyond 5 years, as yet IL-2 has failed to get a product licence in the USA [40]. In contrast granulocyte colony-stimulating factor (G-CSF), though possibly saving the occasional patient from dying of leukopenic sepsis, has been licensed without any evidence that a single extra cancer patient has been cured. The only documented study suggests marginally though non-significant shorter survival and fewer responses in treated patients than in conventionally treated controls [41]. Such a treatment will undoubtedly increase costs of cancer care as clinicians increase cancer chemotherapy dosage in the as yet unproven belief that more patients may be cured.

Even more paradoxical is the current situation in the USA with autologous lymphocyte therapy. This is being marketed as therapy for renal cell cancer based on the results from 45 patients (16 of whom also received vaccination with autologous tumour), none of whom achieved complete remission, and published in a single article as part of a randomised trial [43]. Admittedly, at that time this study was the largest randomised trial published on immunological treatment of renal cell cancer comparing results with symptomatic treatment, something which has not yet been done for IL-2. The only study to date for interferon, involving only a small number of cases, failed to produce any evidence of benefit [42]. Despite the small numbers in the trial of autologous lymphocyte therapy, the trial showed significant prolongation of survival from 113 to 274 days at the 75% point of the two survival curves. This was the latest point, at the time of publication, that the data were mature enough for actual rather than actuarial assessment. On the basis of this information, recruitment to the trial was terminated and this treatment has now been marketed at a price of about US$25 000 per complete treatment, which is nearly five times the price of a course of IL-2. Autologous lymphocyte therapy involves leucophoresis and stimulation of the lymphocytes *in vitro* in a manner designed to reduce the activity of suppressor lymphocytes. It uses an approach that has not been studied in animal models, and the

Table 1. Pooled results of IL-2 with or without IFN-α or LAK for renal cancer

	Single agent	+IFN-α	+LAK cells
Low-dose IL-2 (outpatient)	3+8/56 (20%)	4+27/145 (21%)	ND
High dose IL-2 (inpatient)	15+33/328 (13%)	4+17/105 (20%)	17+37/302 (18%)

ND = not done.
For references see [35].

principal *in vitro* data to support the concept are published only in abstract form [43]. Furthermore, the trial did not control for the placebo effect of repeated leucophoresis without *in vitro* stimulation. Because the process does not involve giving an experimental drug, but only giving irradiated autologous lymphocytes back to the patient after a period of culture, and is relatively free of side-effects (which of course contrasts markedly with most of the publications on IL-2), it has not had to be licensed by the drug regulatory authorities.

The issue of treatment toxicity is one of the principal reasons for the dispute over licensing IL-2. The critical issue has been one of dose/schedule and cost/benefit ratio. All conventional cytotoxic drugs are tested at the maximum tolerated dose. When IL-2 was first investigated this was the approach used, despite the demonstration from IFN-α that this was not the best way to get maximum benefit [44, 45]. Initially 39 patients were treated by multiple different schedules without response [46]. As animal models had demonstrated synergy between IL-2 and *in vitro* IL-2-activated lymphocytes (LAK-49), the two were used in combination at the maximum tolerated dose, using an IL-2 schedule that necessitated intensive care for most patients and had only been tested on a small number of patients. Only after more than 300 patients had been entered into multiple series of phase II studies did it become apparent that there was little difference between high-dose IL-2 alone or combined with LAK [48], a finding which was subsequently confirmed in two randomised trials [49, 50]. Though neither produced clear evidence of benefit for the complicated cell culture process needed to produce LAK cells, there was a suggestion that for melanoma there might be more durable complete remissions in the most mature study [49].

As a consequence of these observations, in renal cell cancer most subsequent studies have focused on combining IL-2 with IFN-α. There has been little effort to get data on single-agent IL-2, particularly using the lower dose outpatient schedules which have been used in many of the combination studies (Table 1).

In the past a wide range of treatments have been tried for renal cell cancer, including hormones, chemotherapy, tumour vaccines, BCG vaccine and IFN-α, before the IL-2 era. They have all generated periods of temporary enthusiasm prior to more balanced scepticism [51]. For chemotherapy agents and hormones, responses have settled at under 5% and few consider they offer any therapeutic advantage (though a recent survey of urologists showed that 80% still use medroxyprogesterone acetate as first-line treatment [52], more because of its temporary steroid appetite-stimulant effect than any likelihood of therapeutic gain). On the basis of the current incidence of death from renal cell cancer, such a practice is probably costing between £100 000 and £300 000 a year in the UK; though because this drug has until now been prescribable through "non-rate-capped" general practioner budgets it has not been controllable.

Our work has shown that it is possible to give IL-2 by the subcutaneous route and achieve equivalent levels of lymphocytosis to that seen with high doses. Level of lymphocytosis is one factor reported by some [56, 57] to correlate with response. Such observations lead to a need to question the value of studies using IL-2 drug dosage at a level of toxicity that necessitates intensive care unit support. However, until equivalent durable complete remissions have been demonstrated, it would be premature to stop such studies all together. Evidence from animal IL-2 dose-response studies [58] supports the low-dose approach since the area under the curve (enhanced by subcutaneous injection) may better relate to the biological effect than does peak dose (more marked with intravenous bolus dosage). There is also evidence from *in vitro* studies that prolongation of stimulus beyond 4 days leads to down-regulation of expression of high-affinity IL-2 receptors [59]. This, with the evidence that there may be a bell-shaped dose-response curve in animal models [60], provides ample justification for more extensive exploration of the lower dose subcutaneous regimens. However, given the large sums invested in getting the drug to this stage (over US$200 million) the issue that needs to be faced is how to offset the costs of further development without some sort of licence. On the basis of today's licencing standards for IL-2, it is doubtful that bleomycin would be licenced for testis cancer given the drug's lethal lung toxicity and less than 5% complete response rate as a single agent [61]. However, recent studies are demonstrating that it is vital to the success of combination therapy [62]. A mechanism is needed to recycle the resources that are spent on provera or criteria to identify funds for developmental clinical trials: this would provide the basis for studies that might ultimately lead to IL-2 contributing to the management of a range of different tumour types. This would be particularly so in the subset of patients with retained expression of HLA class I, given that all the conventional cancer treatment modalities suppress immune reponse [63–65] and both chemotherapy and radiotherapy are often less effective in well differentiated tumours.

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Melanogenesis: a Realistic Target for Antimelanoma Therapy?

P.A. Riley

Melanin is a widely-distributed pigment in the biosphere. In the human adult, the enzymatically-catalysed process of melanin generation is the exclusive prerogative of melanocytes. Melanogenesis generates a number of reactive intermediates including orthoquinones and has been recognised as a potential hazard to melanocytes. Amplification of this cytotoxic hazard to selectively damage malignant melanogenic cells has been investigated as a rational therapeutic strategy for melanoma. A number of surrogate substrates for tyrosinase have been studied, including a range of phenols and catechols. Initial attempts to use these agents for the treatment of disseminated melanoma have foundered on problems due to unfavourable pharmacokinetics, primary toxicity or pharmacological actions of the analogue substrates, and the toxicity of hepatic metabolites. Successful exploitation of the undoubted potential of the metabolic targeting strategy presented by the subversion of melanogenesis depends on the development of prodrugs with minimal primary toxicity and improved pharmacokinetics. The range of possible novel approaches is being extended by the emergent understanding of the complexities of melanogenesis which are outlined.
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MELANINS

MELANINS ARE highly conjugated bathochromic aromatic polymers of uncertain structure. Although melanins are widely distributed in nature, it is not clear what evolutionary pathway has led to their generation. In animals, melanins generally comprise a high proportion of indoles which copolymerise with other residues to give a range of macromolecular pigments [1, 2]. Although the pronounced light and heat-absorbing properties render melanins a potentially important photoprotective, and possibly phonoprotective, pigment in vertebrates, the selective forces acting at earlier stages of evolution are likely to have been connected with the advantages conferred by the initial metabolic steps in its biosynthesis [3].

In mammals indolic melanin is metabolically derived from the aminoacid L-tyrosine by a complex process. The initial oxidation involves ring hydroxylation and subsequent dehydrogenation of tyrosine (see Fig. 1, structure **1**) to form the corresponding L-phenylalanine-3,4-orthoquinone [dihydroxyphenylalanine quinone, or dopaquinone (DQ, structure **3**)]. The enzyme catalysing this oxidation is tyrosinase which, whilst exhibiting stereospecificity with regard to the aminoacid side chain, is able to oxidise a range of analogous phenols and catechols. The corresponding orthoquinones are reactive molecules which readily undergo redox reactions and reductive addition reactions with nucleophiles (Michael addition [4] reactions). The capability of generating reactive quinones with irritant properties is important in defensive sprays of certain arthropods [5] and is probably the physiological basis of the action of the supposed obfuscating ink of cephalopods. Enzymatically-generated orthoquinones are involved in the process of cuticular hardening in insects [6]. Whether there are important functions of melanin precursors that have preserved this metabolic pathway in mammals is not known, but the relatively modest nature of the abnormalities present in albinos, in which mutations of the tyrosinase gene [7] result in greatly reduced levels of quinone generation, give little indication of such a role. Nevertheless, as a general rule, melanogenesis is ubiquitous among vertebrates and is a predominant function of a set of neural crest-derived dendritic cells, the melanocytes.

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